Design and synthesis of novel 1,3,4-oxadiazole based azaspirocycles catalyzed by NaI under mild condition and evaluated their antidiabetic and antibacterial activities

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Abstract
A modest, efficient, and mild synthetic procedure has been developed for the synthesis of novel series of 1,3,4-oxadiazole containing azaspirocycles derivatives. The reaction of 1,3,4-oxadiazole derivative with diverse azaspiro compounds under room temperature condition with helps of sodium iodide catalyst and polar aprotic solvent. Numerous compensations of this strategy embrace less time required, yield increment, consumption of all reactants, mild condition. All synthesized compounds evaluated for *in vitro* antidiabetic and antibacterial screening. Among them some compounds show significant biological response.

Keywords: Azaspirocycles, Mild reaction, 1,3,4-oxadiazole, Antidiabetic, Antibacterial

1. INTRODUCTION

Proficient synthesis of synthetic drug like molecules has been the focal point of the exploration for chemist and biologist because of they play a significant role in drug discovery [1]. Heterocycles containing spiro moieties available in huge number of plants such as lactones, alkaloids, and terpenoids. The substituted spiro compounds have significant importance in pharmaceutical field owing to diverse biological response, numerous synthetic drugs are relying upon spiro nucleus. six-member piperidine fused spiro derivatives used for decrease hypertension [2]. trospium has combined pyrrolidine spiro motif utilized for overactive bladder [3], combination of both diazaspiro[4.5]decan derivatives as potential chitin synthase inhibitors and antifungal agent [4], diazaspiro[5.5]undecane Derivatives use for pain treatment as dual μ-Opioid Receptor Agonists and σ_1 Receptor Antagonists and radioligands for sigma-1 receptors [5-6], also use for treatment of diseases including Huntington's disease (HD), Parkinson's disease (PD), Alzheimer's disease (AD) [7-8]. Some spiro derivatives shows antibacterial activity [9] and antidiabetic activity [10] identical virtuous. Several specific syntheses for different member with dissimilar hetero atom containing spiro scaffolds including bicyclic construction amidines [11], advanced angular spirocycles or liner spirocycles[12], *via* enantioselective hydrogenation [13]. a four-member ring containing spirocycles synthesized by different strategies [14]. Several drugs contain various spirocycles shows in **Fig. 1.**

Another motif oxadiazoles profound class of heterocyclic chemistry that stood out because of various application in medicinal field and synthetic field. oxadiazole based on their isomeric

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form (1,2,3-, 1,2,4-, 1,2,5-, 1,3,4-), among these isomers 1,2,3-oxadiazole is unstable [15], and 1,3,4-oxadiazole derivatives are extremely stable [16]. A plenty of literature, 1,3,4-oxadiazole derivatives implanted in conceivably vigorous molecules [17] Preparation of 1,3,4-oxadiazole several methods have been reported in literature. frequently used synthetic route for preparation of 1,3,4-oxadiazole including reaction between acid hydrazide and acid chlorides, another one directly cyclization of diacylhydrazines by means of various dehydrating agents such as phosphorous pentoxide [18], phosphorous oxychloride [19], thionyl chloride [20], triflic anhydride [21], polyphosphoric acid [22]. reaction of hydrazine with carboxylic acid have been reported with diverse oxidizing agents such as PEG (polyethylene glycol) with dichlorophosphate [23], TBTU [24], Burgess Reagent [25], cyanuric chloride [26], Deoxo-Fluor [27]. 1,3,4-oxadiazole derivatives are reported to shows diverse activities such as anticancer [28], anti-HIV [29], antibacterial [30], antifungal [31-32], anticonvulsant [33], antiviral [34] and antitumor activities [35]. few marketed drugs are shown in **Fig. 2.**

Figure 1. Some marketed drug containing spirocycles

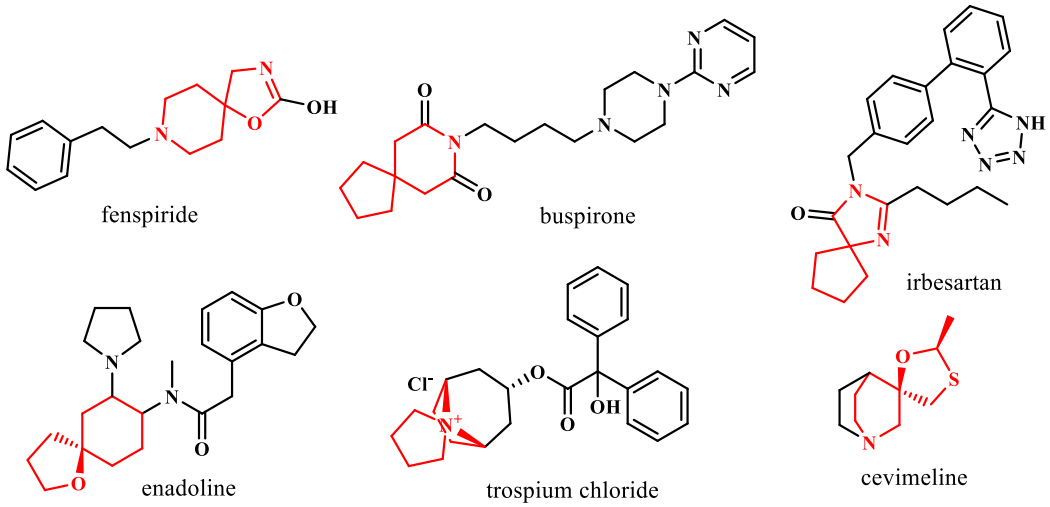
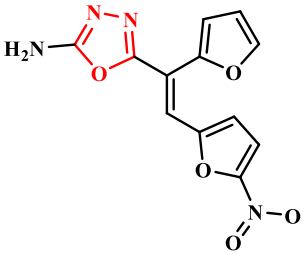
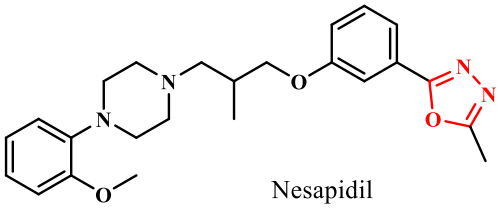


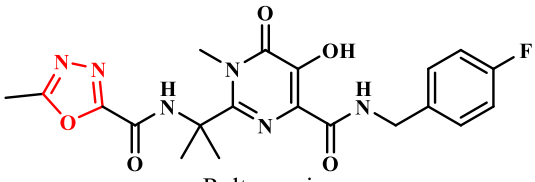
Figure 2. Some marketed drug containing 1,3,4-oxadiazole



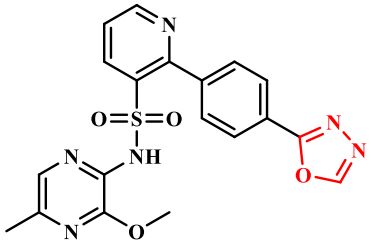
Furamizole



Nesapidil



Raltegravir



Zibotentan

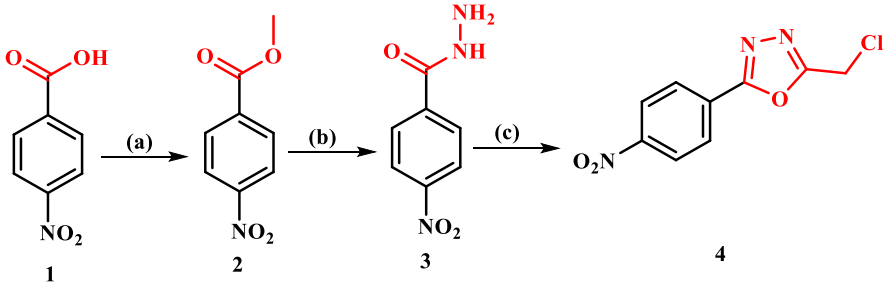
The appearance of bacterial confrontation from the antibiotics signifies a thoughtful apprehension for clinical experts during the most recent decade. Some specific drug resistant including methicilline against *Staphylococcus aureus* [36], and vancomycin resistant *enterococci* gram positive bacteria [37]. in our studies on this article targeted synthesis of antibacterial and antidiabetic dual nature compounds. on review of literature basis, we choose two different pharmacophore 1,3,4-oxadiazole and spirocycles that shows significant desired activities. our designed synthetic tactic we got extremely significant results for antibacterial and antidiabetic activities.

2. RESULTS AND DISCUSSION

2.1 Chemistry

In our preliminary study preparation of 1,3,4-oxadiazole which was prepared by different methods but we choose phosphorous oxychloride method. first, esterification of benzoic acid undergoes H_2SO_4 we got benzoate **(2)**. further it reacts with hydrazine hydride to form hydrazide **(3)** adduct. furthermore, oxidative cyclization of hydrazide by phosphorous oxychloride and chloroacetic acid under solvent free condition for 4 hr reflux to get desired adduct **(4)**. Yield of synthesized compounds were obtained between 80-86%. Synthesized compound 2, 3, and 4 were characterized by ^{1}H NMR spectroscopy and mass spectrometry.

Scheme 1. Preparation of substituted 1,3,4-oxadiazole

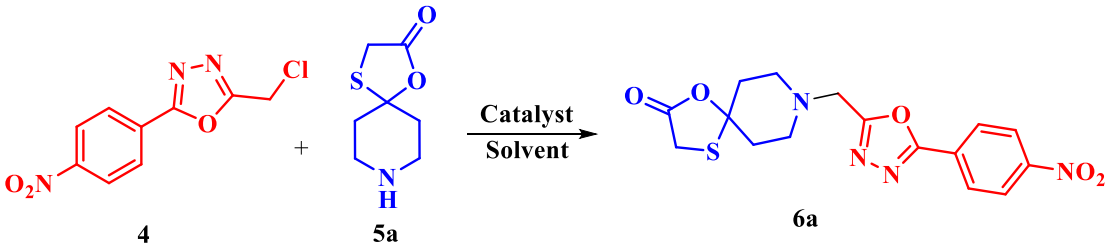


Reaction condition: (a) H_2SO_4, MeOH, reflux, 2h, **(b)** NH_2NH_2, EtOH, 80^0C, 2h, **(c)** $POCl_3$, Chloroacetic acid, 80^0C, reflux 4h.

Now, we move towards the final reaction, we choose 2-(chloromethyl)-5-(4-nitrophenyl)-1,3,4-oxadiazole **(4)**, and 1-oxa-4-thia-8-azaspiro[4.5]decan-2-one **(5a)** has chosen as a model reaction. In the absence of catalyst, we could not obtain desired product in desired amount even after 10 h of stirring in ethanol solvent. We tried to different solvent condition like methanol, DMF, and acetonitrile among them we got some yield increment and satisfactory result in acetonitrile solvent. Furthermore, we choose acetonitrile solvent in all the reaction but this process takes required more time and some less yield as per our expectation. So that we were applied different catalyst in reaction with different mole percentage.

For the preparation of 8-((5-(4-nitrophenyl)-1,3,4-oxadiazol-2-yl)methyl)-1-oxa-4-thia-8-azaspiro[4.5]decan-2-one (6a) first we tried polar protic solvents like ethanol at 50^0C for 10 hr but didn't get exemplary yield also tried methanol but we show yield decrement. So, that our focus moved on polar aprotic solvents like DMF and acetonitrile amongst them acetonitrile gave good yield compare to DMF solvent (Table 1). Synthesized compound didn't give desired yield so, we applied catalyst to enhance reaction rate as well yield increment. Initially we applied 10% mol tetrabutylammonium iodide (TBAI) 46% yield obtained which was much better than without catalyst. After that we used 10% mol Tetra-n-butylammonium bromide (TBAB) and p-Toluenesulfonic acid (*P*-TSA) we got 37% and 21% yield respectively (Table 1). Finally, we done this reaction with sodium iodide catalyst because of it shows higher yield respect to all catalyst. Variation in mole % of sodium iodide like 10%, 15%, 20% and 25% but we got maximum yield with 20% mole of catalyst. All the entries summarized in **Table 1** (entry 1-11). series of azaspirocycles containing 1,3,4-oxadiazole moiety shows in **Scheme 2.**

Table 1. Model reaction and optimization of reaction conditions[a]



4 5a 6a

Entry	Catalyst (mol %)	Reaction condition	Time (h)	Yield (%)[b]
1	-	EtOH, 50^0C	10	23
2	-	MeOH, 50^0C	10	19
3	-	MeCN, 50^0C	10	32
4	-	DMF, 50^0C	10	29
5	TBAI (10%)	MeCN, rt	5	46
6	TBAB (10%)	MeCN, rt	10	37
7	*P*-TSA (10%)	MeCN, rt	10	21
8	NaI (10%)	MeCN, rt	5	49
9	NaI (15%)	MeCN, rt	3	56

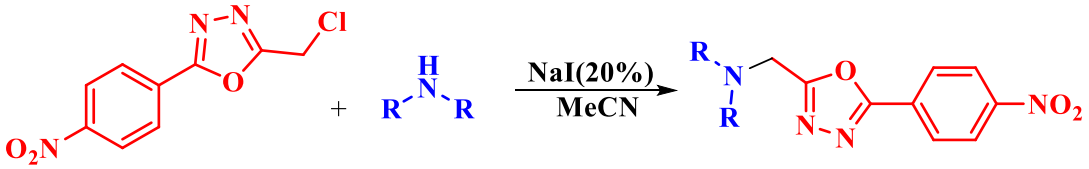
10	***NaI (20%)***	***MeCN, rt***	***3***	***70***
11	NaI (25%)	MeCN, rt	3	65

[a] Reaction conditions: 2-(chloromethyl)-5-(4-nitrophenyl)-1,3,4-oxadiazole (1.71 mmol, 4), 1-oxa-4-thia-8-azaspiro[4.5]decan-2-one (1.9 mmol, 5a), K_2CO_3 (5 mmol) and solvents 6 mL at different temperatures

[b] Isolated yield

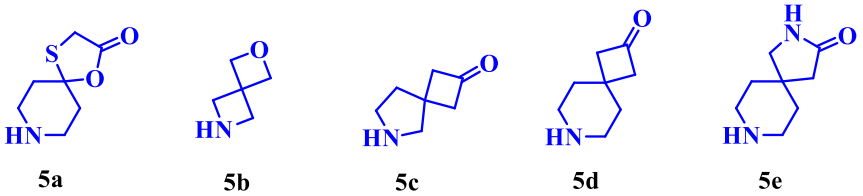
Bold value shows final optimal condition of reaction

Scheme 2. Preparation of azaspirocycles holding 1,3,4-oxadiazole moiety



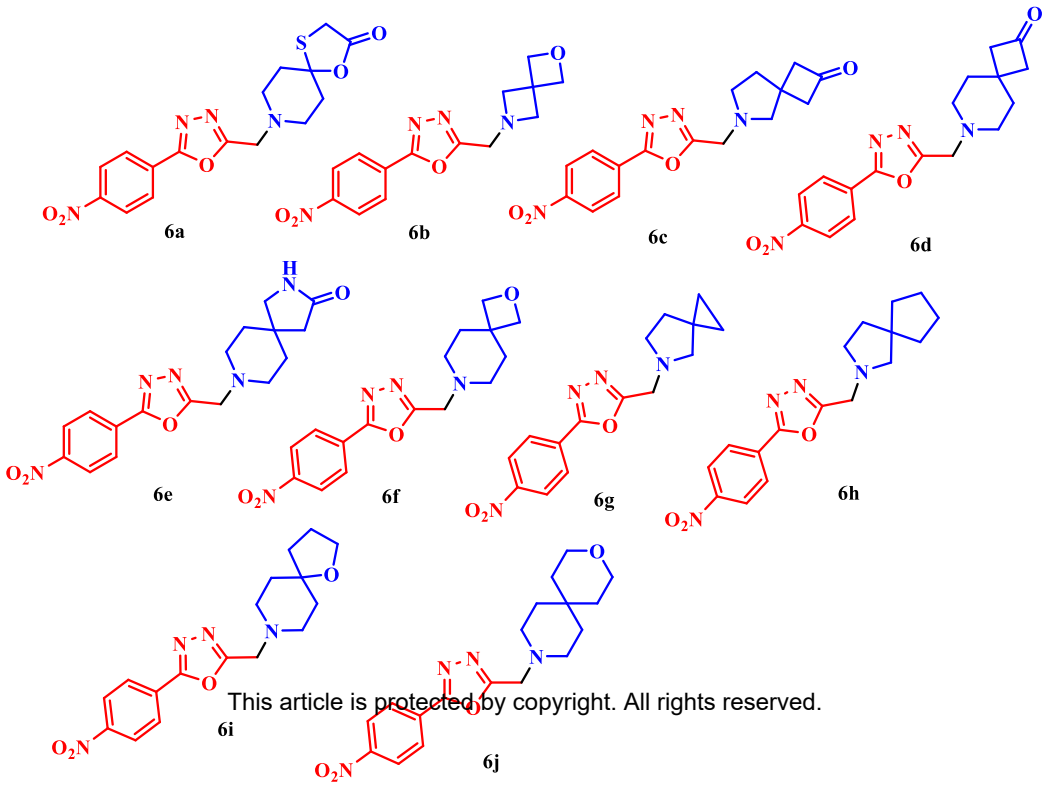
4 5a-j 6a-j

Table 2. Substitution of azaspiro compounds



5a 5b 5c 5d 5e 5f 5g 5h 5i 5j

Table 3. Final synthesized adducts containing azaspiro and 1,3,4-oxadiazole pharmacophore



6a 6b 6c 6d 6e 6f 6g 6h 6i 6j

2.2 Biology

Synthesized compounds **6a-j** tested minimum inhibitory concentration (MIC) determination by the agar well diffusion method [38–40] employed in this work, all synthesized compounds activity checked against Gram-positive and Gram-negative bacteria alike *Bacillus megaterium, Bacillus subtilisis, E. coli*, and *Klebsiella*. yielded larger zone of inhibition in primary screening then secondary screening done with 10 µg/ml, 25 µg/ml, 50 µg/ml concentrations. Results against 50 µg/ml concentration shown in table 2. checked their zone of growth inhibition in millimetre (mm). amongst them compound **6a** was extremely active against gram positive bacteria *Bacillus megaterium, Bacillus subtilisis* and growth inhibition value nearer to standard drug erythromycin. compounds **6b** and **6e** also shows moderate activity compare to erythromycin. gram-negative bacterial strains in secondary screening same compounds **6a**, **6b** and **6e** shows moderate active compare to standard drug tetracycline (**Table 4**).

Table 4. Antibacterial secondary screening of synthesized compounds[a]

Entry	Comp. code	Zones of growth inhibition[b]				
		Gram-positive bacteria		Gram-negative bacteria		
		Bacillus megaterium	*Bacillus subtilisis*	*E. coli*	*Klebsiella*	EC_{50} µg/mL[c]
1	**6a**	**16**	**15**	**11**	**10**	**28.12** ± 0.40
2	**6b**	**13**	**13**	**7**	**9**	**34.61** ± 0.65
3	6c	6	4	0	nt	75.00 ± 1.50
4	6d	7	6	0	nt	64.28 ± 1.25
5	**6e**	**14**	**12**	**11**	**11**	**32.14** ± 0.57
6	6f	10	9	4	8	45.00 ± 1.35
7	6g	9	6	2	7	50.00 ± 1.55
8	6h	8	3	2	4	56.25 ± 1.48
9	6i	10	5	3	6	45.00 ± 1.37
10	6j	4	4	0	nt	112.5 ± 2.32
11	T	nt	nt	17	19	nt
12	E	**18**	**16**	nt	nt	**25.00 ± 0.50**

[a] Sample concentration 50 µg/mL
[b] Zone of growth inhibition measured in millimetre (mm)
[c] EC_{50} concentration for gram positive bacteria *Bacillus megaterium*
T - Tetracycline
E - Erythromycin
0 Value shows no inhibition

nt- Not tested
Bold value shows highly active compounds from synthesized compounds

Synthesized compounds **6a-j** tested for antidiabetic screening by *in vitro* α-amylase inhibitory study. α-amylase inhibitory assay was performed by 3,5-dinitrosalicylic acid (DNSA) method [41]. all synthesized compounds percentage inhibition checked by different sample concentration like 50 µg/mL, 75µg/mL, 100 µg/mL 125 µg/mL. For a reference we used standard drug acarbose, compare the results of both acarbose and synthesized compounds to calculate their IC50 value in µg/mL. among them are 6a, 6b, and 6c shows significantly activity and rest are shows good to moderate activity (**Table 5**). compound 6a highly active and percent of inhibition very close to standard drug acarbose. All the data mentioned in **Table 5**.

Table 5. *in vitro* antidiabetic screening of synthesized compounds.

Entry	Compound	% Inhibition				IC_{50} µg/mL
		50µg/mL	75µg/mL	100µg/mL	125µg/mL	
1	**6a**	**37.91**	**48.23**	**60.09**	**82.21**	**78.07**
2	**6b**	**36.28**	**47.07**	**57.32**	**80.67**	**81.76**
3	6c	34.64	43.60	54.37	76.48	89.64
4	6d	29.19	39.58	47.94	61.29	103.93
5	**6e**	**37.33**	**47.97**	**58.48**	**81.04**	**79.80**
6	6f	27.72	35.81	45.69	60.85	106.86
7	6g	22.51	31.02	40.38	57.36	114.02
8	6h	31.87	40.77	49.56	71.77	100.72
9	6i	32.52	38.46	51.12	73.32	97.28
10	6j	30.78	36.55	50.41	68.78	99.49
11	Acarbose	38.52	49.44	61.18	84.69	75.90

Bold value shows highly active compounds from synthesized compounds

3. CONCLUSION

In summary, we have designed efficient methodology for the synthesis of bioactive and potent heterocycles which was the combinations of two different pharmacophore 1,3,4-oxadiazole and azaspirocycles under the mild reaction condition. synthesized compounds screened for antidiabetic activity and antibacterial activity to get significant results. synthesized molecules will find application in versatile are like medicinal research field and organic chemistry.

4. EXPERIMENTAL

4.1 General

All chemicals, solvents and media were purchased from sigma Aldrich, combi-block, enamine, Himedia, SRL. all purchased chemicals were used without further purification, reactions were continuous monitored by thin layer chromatography (TLC) on silica gel-(G60 F254 (Merck)) of 0.5 mm thickness, visualizing with ultraviolet light (254 and 365nm), or with iodine vapor or aq. KMnO4. Melting points were determined using a Buchi B-540

capillary apparatus. NMR spectra were recorded on a Bruker Advance 400 MHz spectrometer (400 MHz for ^{1}H NMR and 101 MHz for ^{13}C NMR) respectively in solvents like $CDCl_3$, DMSO and chemical shifts are referenced to the solvent residual signals with respect to tetramethylsilane. standard abbreviations are used to represent signals multiplicities for ^{1}H NMR spectrum s - singlet, d - doublet, t - triplet, q - quartet, m - multiplate. Elemental analysis was carried out on Euro EA 3000 elemental analyser and the results are in agreement with the structures assigned. The control of reaction temperature was monitored by ruby thermometer. Mass spectra were recorded on a Shimadzu GC-MS-QP-2010 mass spectrometer in EI (70eV) model using direct inlet probe technique and m/z is reported in atomic units per elementary charge.

4.2 Antibacterial assay

The method is based on the principle that involves the ability of the compound to inhibit the growth of organisms, as exhibited by a clear zone of inhibition [38-40]. The lowest concentration inhibiting the growth of the organism is recorded as the MIC. To check antimicrobial activity, using culture media: nutrient broth, nutrient agar plates. The inoculum was prepared previously. For inoculum preparation, take 50 ml Nutrient broth in a flask and inoculate wire loop culture of bacterial strains. for fungi Bacterial culture incubates at 37 ℃ for 24 hours. All synthesized compounds were dissolved in DMSO (Stock solution:2000 µg/ml concentration). For primary screening, prepare three dilutions 1000 µg/ml, 500 µg/ml and 250 µg/ml from the stock solution of synthesized compounds. To check MIC prepared nutrient agar plate and allowed to solidify. after solidification, bacterial strains were spread on to the solidify plates by spread plate technique and make well on the agar plates by using a 7mm cupbearer. After preparation of well, synthesized compound dilutions (1000 µg/ml, 500 µg/ml and 250 µg/ml) were added 100 µl into the well of plates. These bacterial plates were incubated at 37^{0}C for 24 hours. The diameter of zone of inhibition extending laterally around the wells were measured. For secondary screening, the synthesized compound found active in primary screening were similarly diluted to obtain 200 µg/ml 10 µg/ml, 25 µg/ml, 50 µg/ml concentrations. The diameter of the zone of inhibition was measured in millimetre (mm).

4.3 In vitro α-Amylase Inhibitory study

The α-Amylase inhibition assay was performed using the 3,5-dinitrosalicylic acid (DNSA) method. All the compounds were dissolved in 10% DMSO and were further dissolved in buffer at pH 6.9 to give concentrations ranging from 10-1000 µg/ml. A volume of 200 ml of α-amylase solution (2 units/ml) was mixed with 200µl of the dissolved compounds and was incubated for 10 minutes at 30°C.There after 200µl of starch solution (1% in water (w/v)) was added to each tube and incubated for 3 minutes. The reaction was terminated by the addition of 200µl DNSA reagent (12gm of sodium potassium tartrate tetra hydrate in 8.0ml of 2 M NaOH and 20ml of 96 mM of 3,5-dinitrosalicylic acid solution) and was boiled for 10 minutes in a water bath at 85-90°C. The mixture was cooled to ambient temperature and was diluted with 5ml of distilled water, and the absorbance was measured at 540 nm using UV-Visible spectrometer. The blank with 100% enzyme activity was prepared by replacing the dissolved compounds with 200 µL of buffer. A blank reaction was similarly prepared using the dissolved compounds at each concentration in the absence of enzyme solution. A positive

control was prepared using acarbose (125µg/mL-10µg/mL) and the reaction was performed similarly to the reaction with dissolved compounds as mentioned above. The α-amylase inhibitory activity was expressed as percent inhibition and was calculated using the equation given below: The % of a-Amylase inhibition was plotted against the concentration of dissolved compounds and calculated their IC50 (µg/mL) value respectively [41].

$$\% \text{ of } \alpha \text{ amylase inhibition} = 100\times \text{Abs}_{100\%\ \text{control}}\text{-Abs}_{\ \text{sample}} / \text{Abs}_{100\%\ \text{control}}$$

Procedure for the synthesis of methyl 4-nitrobenzoate (2).

To a stir solution of 4-nitro benzoic acid (89.75 mmol) in Methanol (150 ml) was added sulphuric acid and reaction mixture was reflux at 60^0C for 2h, Progress of reaction was monitored by TLC, After completion of reaction, reaction mixture was concentrated and poured into ice cold water (400 ml), solid material was precipitate out which was filtered out and dried u/vacuum to get methyl-4-nitrobenzoate **(2).**

Methyl 4-nitrobenzoate **(2)** Yield: 85.04% (14.0 g) as an off-white solid material. Mp: 94-96^0C. ^{1}H NMR (400 MHz, DMSO) δ 8.36 (d, *J*=8.4 Hz, 2H), 8.20 (d, *J*=8.8 Hz, 2H), 3.92 (s, 3H), Mass m/z: 181.15. Elemental Analysis: $C_7H_8NO_4$, Calculated: C, 53.04; H, 3.90; N, 7.73; O, 35.33. Found: C, 53.14; H, 3.75; N, 7.53.

Procedure for the synthesis of 4-nitrobenzohydrazide (3).

To a stir solution of methyl-4-nitrobenzoate (71.76 mmol) in Ethanol (130 ml) was added hydrazine hydrate (143.52 mmol) and reaction mixture was reflux at 78^0C for 2h, Progress of reaction was monitored by TLC, After completion of reaction, reaction mixture was concentrated and poured into ice cold water (200 ml), solid material was precipitate out which was filtered out washed with water and dried u/vacuum to get 4-nitrobenzo hydrazide **(3).**

4-nitrobenzohydrazide **(3)** Yield: 84.61 % (11.0 g) as a light-yellow solid material. Mp: 212-214^0C. ^{1}H NMR (400 MHz, DMSO) δ: 10.14 (s, 1H), 8.30 (d, *J*=8.8 Hz, 2H), 8.04 (d, *J*=8.4 Hz, 2H), 4.65 (s, 2H), Mass m/z: 181.15.Elemental Analysis: $C_7H_7N_3O_3$, Calculated: C, 46.41; H, 3.90; N, 23.20; O, 26.50. Found: C, 46.25; H, 3.70; N, 23.26.

Procedure for the synthesis of 2-(chloromethyl)-5-(4-nitrophenyl)-1,3,4-oxadiazole (4).

To a stir solution of Phosphoryl chloride (80 ml), 4-nitrobenzo hydrazide (44.16 mmol) and chloroacetic acid (44.16 mmol) were added at 0^0C, then reaction mixture was reflux at 80^0C for 4h, Progress of reaction was monitored by TLC, After completion of reaction, reaction mixture was concentrated and poured into ice cold water (200 ml), solid material was precipitate out which was filtered out washed with water and dried u/vacuum to get 2-(chloromethyl)-5-(4-nitrophenyl)-1,3,4-oxadiazole **(4)**

2-(chloromethyl)-5-(4-nitrophenyl)-1,3,4-oxadiazole **(4)** Yield: 86.71 % (6.0 g) as an off-white material, Mp: 134-136^0C. ^{1}H NMR (400 MHz, DMSO) δ: 8.45 (d, *J*=8.4 Hz, 2H), 8.29 (d, *J*=8.8 Hz, 2H), 5.19 (s, 2H), Mass m/z: 239.62, Elemental Analysis: $C_9H_6ClN_3O_3$, Calculated: C, 45.11; H, 2.52; Cl, 14.79; N, 17.54; O, 20.03. Found: C, 45.18; H, 2.56; Cl, 14.69; N, 17.34.

General procedure for the synthesis of 1,3,4-oxadiazole containing azaspirocycles (6a-j)

To a stir solution of azaspirocycles derivatives **(6a-j)** (1.90 mmol) in Acetonitrile (6 ml) K_2CO_3 (5.72 mmol) was added and reaction mixture was stirred at rt for 15 min then 2-(chloromethyl)-5-(4-nitrophenyl)-1,3,4-oxadiazole (1.71 mmol) and NaI (20 mol%) were added then reaction mixture was stirred at rt for 3h, Progress of reaction was monitored by TLC, After completion of reaction, reaction mixture was poured into water (10 ml) and extracted with EtOAc (10-20 ml), combined organic layer was dried over Na_2SO_4,concentrated u/vacuum to get crude material which was purified by column chromatography using 30% Ethyl acetate/n-Hexane as a mobile phase to get pure compounds **(6a-j)**.

8-((5-(4-nitrophenyl)-1,3,4-oxadiazol-2-yl)methyl)-1-oxa-4-thia-8-azaspiro[4.5]decan-2-one **(6a).** Yield**:** 70.02% (0.320 g) as a light brown solid material, Mp: 180-182^{0}C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.43 (d, *J*=8.8 Hz, 2H), 8.32 (d, *J*=8.8 Hz, 2H), 3.99 (s, 2H), 3.79 (s, 2H), 2.87 (d, *J*=8.0 Hz, 2H), 2.80 (d, *J*=4.4 Hz, 2H), 2.29 (d, *J*=13.6 Hz, 2H), 2.16 (d, *J*=8.4 Hz, 2H), ^{13}C NMR (101 MHz, $CDCl_3$) δ: 171.74, 164.31, 163.75, 149.70, 129.21, 127.99, 124.40, 89.49, 51.61, 50.29, 39.14, 31.82, 29.69, Mass m/z: 376.39, Elemental Analysis: $C_{16}H_{16}N_4O_5S$, calculated: C, 51.06; H, 4.28; N, 14.89; O, 21.25; S, 8.52, Found: C, 51.16; H, 4.22; N, 14.80.

6-((5-(4-nitrophenyl)-1,3,4-oxadiazol-2-yl)methyl)-2-oxa-6-azaspiro[3.3]heptane **(6b).** Yield: 65% (0.24 g) as a light yellow solid, Mp: 176-178^{0}C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.40 (d, *J*=8.8 Hz, 2H), 8.28 (d, *J=8.8* Hz, 2H), 4.79 (s, 4H), 3.92 (s, 2H), 3.65 (s, 4H), ^{13}C NMR (101 MHz, $CDCl_3$) δ: 172.89, 162.13, 147.90, 129.27, 128.09, 125.25, 77.37, 76.74, 63.17, 62.21, 50.16, 32.25. Mass m/z: 302.29, Elemental Analysis: $C_{14}H_{14}N_4O_4$, calculated: C, 55.63; H, 4.67; N, 18.53; O, 21.17 Found: C, 55.56; H, 4.62; N, 18.43.

6-((5-(4-nitrophenyl)-1,3,4-oxadiazol-2-yl)methyl)-6-azaspiro[3.4]octan-2-one **(6C).** Yield: 76.22% (0.300 g) as a brown solid material. Mp: 198-200^{0}C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.43 (d, *J*=9.2 Hz, 2H), 8.31 (d, *J*=8.8 Hz, 2H), 4.12 (s, 2H), 3.19 (s, 2H), 3.13 (t, *J*=3.6 Hz, 2H), 3.07 (s, 4H), 2.22 (t, *J*=7.2 Hz, 2H), ^{13}C NMR (101 MHz, $CDCl_3$) δ: 200.19, 184.34, 176.87, 167.49, 145.72, 134.48, 129.62, 126.50, 70.07, 64.52, 56.87, 52.30, 39.85. Mass m/z: 328.33, Elemental Analysis: $C_{16}H_{16}N_4O_4$, calculated: C, 58.53; H, 4.91; N, 17.06; O, 19.49, Found: C, 58.43; H, 4.90; N, 17.26.

7-((5-(4-nitrophenyl)-1,3,4-oxadiazol-2-yl)methyl)-7-azaspiro[3.5]nonan-2-one **(6d).** Yield: 67.74% (0.200 g) as a creamish solid material. Mp:178-180^{0}C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.41 (d, *J*=8.8 Hz, 2H), 8.30 (d, *J*=8.8 Hz, 2H), 3.94 (s, 2H), 2.81 (s, 4H), 2.64 (s, 4H), 1.85 (s, 4H). ^{13}C NMR (101 MHz, $CDCl_3$) δ: 202.32, 176.02, 166.62, 159.41, 137.15, 132.30, 128.91, 127.12, 124. 08, 74.84, 63.69, 57.27, 51.04, 46.34, 37.57, 34.19. Mass m/z: 342.35, Elemental Analysis: $C_{17}H_{18}N_4O_4$, calculated: C, 59.64; H, 5.30; N, 16.37; O, 18.69, Found: C, 59.54; H, 5.10; N, 16.32.

8-((5-(4-nitrophenyl)-1,3,4-oxadiazol-2-yl)methyl)-2,8-diazaspiro[4.5]decan-3-one **(6e).** Yield: 70.59% (0.180 g) as a creamish solid material. Mp: 166-168^{0}C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.41 (d, *J*=8.8 Hz, 2H), 8.30 (d, *J*=8.8 Hz, 2H), 3.96 (s, 2H), 3.23 (d, *J*=1.2 Hz,

2H), 2.71 (m, 2H), 2.60 (m, 2H), 2.25 (s, 2H), 1.81 (t, *J*=5.6 Hz, 4H), ^{13}C NMR (101 MHz, $CDCl_3$) δ: 175.61, 172.79, 165.29, 151.43, 137.34, 133.59, 129.47, 127.35, 57.04, 54.83, 50.16, 47.30, 37.46, 36.23. Mass m/z: 357.37, Elemental Analysis: $C_{17}H_{19}N_5O_4$, calculated: C, 57.14; H, 5.36; N, 19.60; O, 17.91, Found: C, 57.24; H, 5.30; N, 19.65.

7-((5-(4-nitrophenyl)-1,3,4-oxadiazol-2-yl)methyl)-2-oxa-7-azaspiro[3.5]nonane **(6f).** Yield: 84.67% (0.440 g) as a light yellow solid material. Mp: 208-210^{0}C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.43 (d, *J*=8.8 Hz, 2H), 8.31 (d, *J*=8.8 Hz, 2H), 4.45 (s, 4H), 3.92 (s, 2H), 2.57 (m, 4H), 1.98 (t, *J*=5.2 Hz, 4H), ^{13}C NMR (101 MHz, $CDCl_3$) δ: 164.57, 163.66, 149.63, 129.28, 127.96, 124.39, 81.57, 52.21, 50.45, 38.06, 34.68. Mass m/z: 330.34, Elemental Analysis: $C_{16}H_{18}N_4O_4$, calculated: C, 58.17; H, 5.49; N, 16.96; O, 19.37, Found: C, 58.12; H, 5.40; N, 16.86.

2-((5-azaspiro [2.4] heptan-5-yl)methyl)-5-(4-nitrophenyl)-1,3,4-oxadiazole **(6g).** Yield: 75.44% (0.350 g) as a creamish solid material. Mp: 194-196^{0}C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.42 (d, *J*=8.8 Hz, 2H), 8.33 (d, *J*=8.8 Hz, 2H), 4.11 (s, 2H), 3.03 (t, *J*=6.8 Hz, 2H), 2.78 (s, 2H), 1.95 (t, *J*=6.8 Hz, 2H), 0.66 (t, *J*=8.4 Hz, 4H), ^{13}C NMR (101 MHz, $CDCl_3$) δ: 172.03, 163.84, 148.62, 134.82, 128.82, 126.24, 67.48, 52.44, 39.51, 13.87. Mass m/z: 300.32, Elemental Analysis: $C_{15}H_{16}N_4O_3$, calculated: C, 59.99; H, 5.37; N, 18.66; O, 15.98, Found: C, 59.90; H, 5.32; N, 18.60.

2-((2-azaspiro [4.4] nonan-2-yl)methyl)-5-(4-nitrophenyl)-1,3,4-oxadiazole **(6h).** Yield: 78.44% (0.350 g) as a yellow solid material. Mp: 186-188^{0}C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.42 (d, *J*=8.8 Hz, 2H), 8.32 (d, *J*=8.8 Hz, 2H), 4.08 (s, 2H), 2.90 (m, 2H), 2.70 (m, 2H), 1.84 (t, *J*=7.2 Hz, 2H), 1.65 (m, 8H). ^{13}C NMR (101 MHz, $CDCl_3$) δ: 173.52, 164.81, 146.86, 134.26, 126.05, 123.18, 67.48, 52.68, 50.65, 42.09, 26.37. Mass m/z: 328.37, Elemental Analysis: $C_{17}H_{20}N_4O_3$, calculated: C, 62.18; H, 6.14; N, 17.06; O, 14.62, Found: C, 62.10; H, 6.12; N, 17.16.

8-((5-(4-nitrophenyl)-1,3,4-oxadiazol-2-yl)methyl)-1-oxa-8-azaspiro[4.5]decane **(6i).** Yield: 75.50% (0.350 g) as a creamish solid material. Mp: 178-180^{0}C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.42 (d, *J*=8.8 Hz, 2H), 8.32 (d, *J*=8.8 Hz, 2H), 3.98 (s, 2H), 3.87 (t, *J*=6.8 Hz, 2H), 2.73 (m, 4H), 1.97 (t, *J*=6.8 Hz, 2H), 1.75 (t, *J*=6.8 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ: 174.65, 162.87, 150.08, 136.47, 130.48, 127.82, 86.79, 71.04, 54.69, 51.38, 38.38, 32.83, 29.82. Mass m/z: 344.37, Elemental Analysis: $C_{17}H_{20}N_4O_4$, calculated: C, 59.29; H, 5.85; N, 16.27; O, 18.58, Found: C, 59.20; H, 5.80; N, 16.25.

9-((5-(4-nitrophenyl)-1,3,4-oxadiazol-2-yl)methyl)-3-oxa-9-azaspiro[5.5]undecane **(6j).** Yield: 81.18% (0.300 g) as a light yellow solid material. Mp: 188-190^{0}C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.42 (d, *J*=8.8 Hz, 2H), 8.32 (d, *J*=8.8 Hz, 2H), 3.98 (s, 2H), 3.69 (t, *J*=5.2 Hz, 4H), 2.67 (m, 4H), 1.69 (m, 4H), 1.61 (m, 4H). ^{13}C NMR (101 MHz, $CDCl_3$) δ: 172.01, 164.43, 150.87, 136.82, 130.41, 125.18, 68.53, 48.47, 37.41, 32.90. Mass m/z: 358.40, Elemental Analysis: $C_{18}H_{22}N_4O_4$, calculated: C, 60.32; H, 6.19; N, 15.63; O, 17.86 Found: C, 60.22; H, 6.13; N, 15.60.

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SUPPORTING INFORMATION

Experimental of synthesis and biological activity, ^{1}H NMR, ^{13}C NMR spectra and Mass spectra for all compounds were provided as Supplementary material.

DISCLOSURE STATEMENT

No potential conflict of interest was reported by the authors.

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